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APPLICATION NUMBER: *60/429,550*
FILING DATE: *November 29, 2002*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 CFR 1.53 (b)(2).

Docket Number	25395	Type a plus sign (+) inside this box ->	

60/429550

INVENTOR(s) / APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
BRAUN	Sergei		Zur Hadassa, Israel

TITLE OF THE INVENTION (280 characters max)

PROCESS FOR PREPARATION OF BIODEGRADABLE PLASTICS FROM PROTEIN AND PLASTICS OBTAINED THEREBY

CORRESPONDENCE ADDRESS

G. E. EHRLICH (1995) LTD.
c/o ANTHONY CASTORINA
2001 JEFFERSON DAVIS HIGHWAY
SUITE 207

STATE	VIRGINIA	ZIP CODE	22202	COUNTRY	USA

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ **Specification**	*Number of Pages*	17	☑ **Applicant is entitled to Small Entity Status**
☐ **Drawing(s)**	*Number of Sheets*		☑ **Other** (specify) *ASSIGNMENT*

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☐ A check or money order is enclosed to cover the filing fees		**FILING FEE AMOUNT ($)**	**$ 80.-**
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No

☐ Yes, the name of the US Government agency and the Government contract number are: ______________

Respectfully submitted,

SIGNATURE ______________________ Date: 27 November 2002

REGISTRATION NO. *(if appropriate)*: 25,457

TYPED or PRINTED NAME SOL SHEINBEIN

☐ Additional inventors are being named on separately numbered sheets attached hereto

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROCESS FOR PREPARATION OF BIODEGRADABLE PLASTICS FROM PROTEIN AND PLASTICS OBTAINED THEREBY

Inventor: Sergei Braun

1. Summary of the Invention

This invention provides a general method of production of biodegradable plastic materials from protein. Undigested protein, protein fragments, small peptides, amino acids or mixtures of thereof are deaminated in a reaction with nitrous acid or with nitrogen oxides. As the result, all or the majority of alpha-amino groups (Fig. 1) present in the source material as well as of the omega-amino groups of lysine and to some extent arginine will be replaced with hydroxyls.

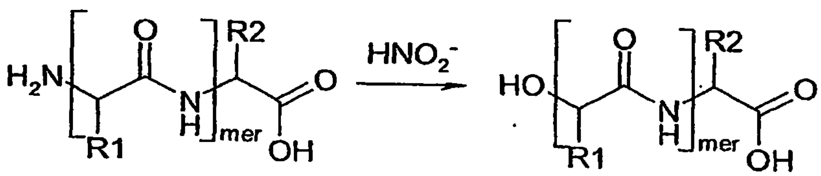

Fig. 1. Deamination of alpha-amino groups with nitrous acid

The protein source also contains hydroxylic groups in the side chains of serine and threonine, carboxylic residues of aspatic and glutamic acids as well as the carboxylic termini of polypeptides and individual amino acids. All these functional group are transferred to the hydroxy-carboxylic protomers resulting from deamination. These protomers are recovered from the reaction medium by extraction or drying and subjected to poly-condensation in the presence of a catalyst.

Thus a variety of branched (Fig. 2) polymer resins of desired molecular mass (e.g., such as of about 50,000-100,000) and viscosity is obtained. The properties of these resins are determined by degree of polymerization and the content of polyamidic component, which can vary from micromeric (obtained from highly digested protein) to macromeric (obtained from poorly digested protein).

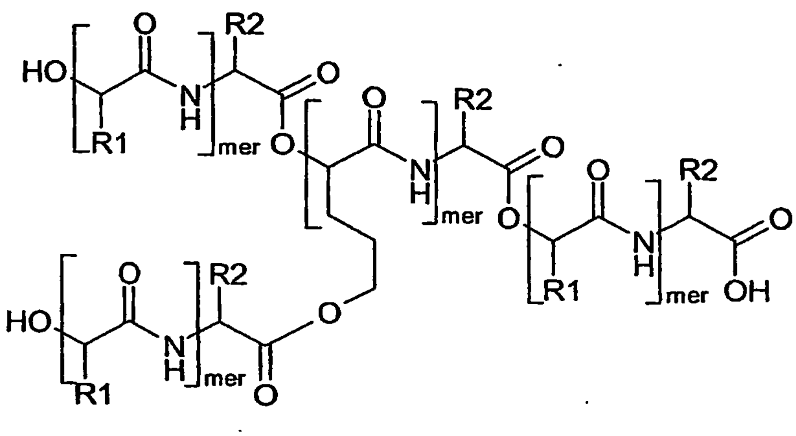

Fig. 2. Branching point formed by the condensation of lysine-derived side chain with carboxy-terminal

Both polyamide and polyester bonds are susceptible to biodegradation, which is aided by irregular non-crystalline structure of the polymer with its variety of side chains and branching points. The rate of biodegradation and the moisture resistance of the polymer can be regulated by the ratio of ester to amide bonds in the polymer. High content of more hydrophobic polyester bonds provides for better moisture resistance.

2. Background of the Invention

Disposal of solid waste has become a major environmental problem resulting in regulatory controls to limit its production and disposal. A major concern is the disposal of environmentally stable plastics used extensively in agricultural films, packaging and many other applications. Concern over non-degradable plastics has resulted in worldwide prohibition of their disposal at sea, increasingly severe regulatory limits and incentives to reduce the amount of non-biodegradable plastics reaching the landfills. These trends have resulted in increased industrial interest in the development and manufacture of biodegradable materials.

Market applications of biodegradable plastics include:

1. Consumer products such as packaging materials, garbage bags and disposable plates.
2. Agricultural products such as a agricultural film for row coverings, where these coverings biodegrade into the soil and do not obstruct machinery during harvesting, and

To date the acceptability of biodegradable plastics has been very limited due to their high cost, which is determined by the cost of the high-purity raw materials required. The novelty of the proposed process is in the potential use of any protein including the inexpensive byproduct streams. Examples of such raw material sources, from which the plastic is produced, include corn gluten, soy flour, slaughterhouse and fish waste, and

even microbial sludge. The proposed process *converts the protein component* of these byproduct streams into biodegradable plastics.

Proteins as natural biodegradable polymers have been sought as starting materials for the preparation of plastics since as early as the end of 19th century. Adolf Spitteler obtained in 1899 a patent for one of the first plastics, whose trade name was Galalith. The basis of this invention was a mixture of casein and formaldehyde. Soon Galalith was used for many domestic items such as low voltage electric plugs and sockets but also fancy goods like jewellery, manicure sets, fountain pens and buttons. In the late 1940's, casein plastics were gradually replaced by more modern plastics.

In general, pure protein materials are fragile and absorb moisture from the environment. Improvement in the mechanical and barrier properties requires protein modification with synthetic organic chemicals to produce hydrophobic protein. Several organic chemicals were used to increase hydrophobicity (to decrease affinity towards water) and to develop grafting sites for the oxidized polyethylene. Thus, formaldehyde and dimethoxy dimethyl silane improved the protein hydrophobicity[1].

Given the availability at a low price of soy, some attempts have been made to directly produce plastics from soy protein. A group led by Prof. J. Jane at Iowa State University has made starting from purified soy protein several biodegradable plastics suitable for application as agricultural mulch film[2]. The film is produced by crosslinking soy protein with aldehyde starch. The primary obstacle to commercialization of these processes is the high cost of purified soy protein comparable to that of polyethylene mulch.

As shown above there are no general methods of producing quality plastic materials from bulk inexpensive proteins in current industrial use, which alone demonstrates the limitations of the existing methods.

The principal, currently available biodegradable plastics fall into three main categories:

- *Polylactides (PLA)*
- *Starch-based polymers*
- *Polyhydroxybutyrate (PHB) / Polyhydroxyvalerate (PHV)*

Polylactides are produced by polymerizing lactic acid in a number of steps. First it is oligomerized to a linear chain by removing water. This oligomer (also known as polylactic acid or PLA) is then de-polymerized to lactide, a cyclic dimer. This six-membered ring is purified and then subjected to ring-opening polymerization in order to produce polylactide. Cargill, Inc. (USA) is the largest producer of polylactide resins. Polylactides can be thermoformed, injection molded or even used as spun fibers. Applications include coatings for paper plates, etc. Lactic acid produces linear polymers and every impurity - every molecule carrying either a hydroxy or a carboxylic group -

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[1] Ghorpade, V.M., Lin, H., and Hanna, M.A. 1993. Physical and mechanical properties of protein-polyethylene extrudate. ASAE Paper No. 93-6533, Chicago, Dec. 1993. Lin, H., Hanna, M.A., and Ghorpade, V.M., 1993. Effects of chemical modifications on soy and wheat protein films. ASAE Paper No. 93-6529, Chicago, Dec. 1993.

[2] USP 5,397,834, USP 5,523,293, USP 5,710,190

results in termination of polymerization and, thus, in low molecular weight products with impaired chemical and mechanical stability.

The main *starch-based polymers* are starch/ethylene-vinyl alcohol copolymer blends which are prepared by blending a starch-based component and an ethylene-vinyl copolymer in an extruder in the presence of water or a plasticizer. The temperature and pressure conditions cause the starch to become destructurized, and the resulting composition forms a thermoplastic melt. These polymers have physical properties similar to polystyrene or polyethylene, however they are sensitive to moisture. Novamont (Italy) produces a starch-based resin (Mater-Bi) suitable for manufacturing injection molded pieces, films (for bags) and loose fill packaging material. Novon International (USA) produces Novon, a starch-based resin for mixing with synthetic polymers, in its New Jersey facility.

Polyhydroxybutyrate (PHB) and *Polyhydroxyvalerate (PHV)* based microbial plastics are currently manufactured by Monsanto in Europe for a few limited niche markets. The technology of producing PHB/PHV based plastics is described in[3].

3. Description of the Invention

The steps involved in the proposed process of converting protein into plastics are detailed in the Table below:

Step	Description
Enzymatic or chemical hydrolysis	Optional: Production of protein fragments, peptides or amino acid solution from protein
Conversion	Conversion of α-amino & side chain amino groups into hydroxylic groups
Recovery	Recovery of hydroxy-carboxylic protomers from water solution
Polycondensation[*]	One-step or two-step condensation of protomers to obtain resins
Formulation	Formulation of resins into a plastic.

3.1. *Enzymatic or chemical hydrolysis of protein*

The process of this invention allows for the use undigested soluble protein. However, many inexpensive protein sources (e.g., glutens) offer mainly or partly insoluble protein. These sources may be enriched in protein and separated from the carbohydrate component by protein hydrolysis. Higher degree of fragmentation of the source protein provides for increased content of more hydrophobic ester bonds in the polymer, and, thus for better moisture resitance. The ratio between the polyester and polyamide component will also influence mechanical properties of the polymer.

[3] Doi, Y. Microbial Polyesters. VCH Publishers, NY, 1990

[*] It is highly probable that a single condensation-polymerization step will be sufficient.

Production of protein hydrolysates, either enzymatic or chemical is a well-known process and needs no special description. The proposed process is best suited by the enzymatic process, which allows to control the size of fragments by selecting proteases of different specificity as well as their concentration, the temperature of the process and its duration.

3.2. *Conversion*

During this step, the side amino groups and the α-amino groups of the protein or the protein digest are replaced with hydroxyls the action of nitrous acid or nitrous oxide. The process will be preferentially carried out at the ambient temperature at the pH values between 3 and 5. The pH will be adjusted by the addition of a hidroxy-carboxylic acid such as l-lactic or glycolic.

Deamination of Amino-Acids and Peptides

P. Walden[4] was the first to describe deamination of asparagine with nitrous oxide, a reaction involving diazonium ion, and resulting in l-malic acid (Fig. 3). He has also demonstrated that this reaction is stereospecific

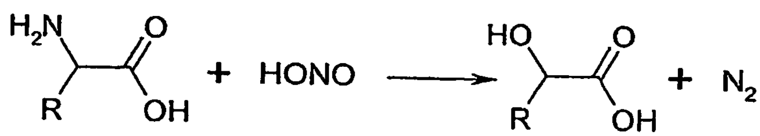
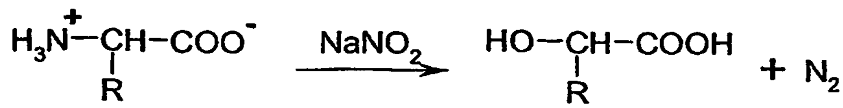


Fig. 3. Deamination of amino acids: General reaction

Nitrous acid ($NaNO_2$ in acidic media) easily transforms amino groups into hydroxyl group with the liberation of one mole of nitrogen. Van Slyke[5] showed that the majority of amino acids when treated with nitrous acid yield their nitrogen quantitatively at room temperature. The reaction has been used mainly as an analytical tool.

In amino acids, the reactions of side chains should be considered. No reaction is expected to take place between the side chains of the aliphatic amino-acids (Gly, Ala, Val, Leu, Ile) and nitrous acid at $pH \approx 3-4$. The $C^{\alpha}-NH_2$ group will react with the acid to form hydroxy-acid (Fig. 4) via diazotation and liberation of N_2.

[4] Walden, P. (1895) Ber. 28, 2767-2773.
[5] Van Slyke, D.D. (1911) J. Biol. Chem. 9, 185.

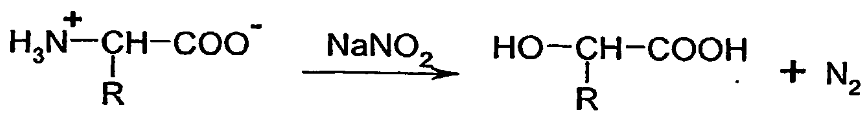


Gly, R=H
Ala, $R=CH_3$
Val, $R=CH(CH_3)_2$
Leu, $R=CH_2CH(CH_3)_2$
Ile, $R=CH(CH_3)(CH_2CH_3)$

Fig.4. Deamination of Glu, Ala, Val, Leu, Ile

The yield in the deamination of Gly is 100%[6].

The carboxylic residue of the two acidic amino acids, Asp and Glu, does not react with nitrous acid (Fig. 5). Therefore, the reaction will proceed with the same mechanism as in aliphatic amino-acids, and the products are hydroxy-di-carboxylic acids.



Asp, $R=CH_2COOH$
Glu, $R=CH_2CH_2COOH$

Fig. 5. Deamination Asp and Glu

Deamination of Glu yields 100%[6]. Depending upon the conditions of the reaction, the ω-amides of Asn and Gln could also be hydrolysed. The ω-amino group of Lys is readily deaminated to hydroxyl. The guanidine group of Arg slowly reacts with nitrous acid liberating nitrogen.

Van Slyke has demonstrated that Cis and Tyr are lost, probably, oxidized by nitrous acid, especially at elevated temperature (45°C). In both amino acids, some nitrosylation was suggested as demonstrated by a yellow-red color of the product observed by Schmidt[7]. Obviously, the products of deamination have to be thoroughly reduced to eliminate nitroso-derivatives.

At 45°C, both Van Slyke and Schmidt have obseved slight degradation of some peptide bonds.

For our process, the optimal conditions producing minimal side chain reactions, except for deamination are at the slightly acidic pH (pH = 3-5) and room temperature or below. In our experiment conducted at the pH = 4 with both protein (BSA) and protein digest (Casamino acids, DIFCO), the theoretical amount of nitrogen was released.

[6] Gouesnard, J. P. (1989). Reactivite du nitrite de sodium. V. Action sur les amino-acides, peptides et proteines. *Bull. Soc. Chim. Fr.* 1, 88-94.
[7] Schmidt, C.L.A. (1929) j. Biol. Chem. 82, 587-594.

3.2. *Recovery of hydroxy-carboxylic protomers*

The product of deamination is a mixture of hydroxy-carboxylic protomers ranging from oligo-peptide derivatives to individual hydroxycarboxylic acids. The common feature of all the protomers is their hydroxy-carboxylic character and, thus, negative charge. Therefore their chemical and extractive properties remind of that of hydroxy-carboxylic acids such as l-lactic acid.

Hydroxy-carboxylic acids such as l-malic acid and l-lactic are natural products of fermentation. Because of their economic value, many methods are known for their separation (extraction)[8]. The same protocols used for the extraction of the fermentation product can be used here in order to separate the hydroxy-carboxylic protomers from the byproducts of the convertion.

Liquid-Liquid extraction with tertiary amines

Tertiary amines are widely used for the extraction and recovery of carboxylic acids from water[9]. One popular example is the use of Alamine 336, a commercial mixture of tertiary amines with C_8, C_{10}, and C_{12} alkyl groups dominant[10]. This is an ion-exchange reaction, because the amine is protonated in the acidic solution and forms an ion pair with the carboxylate ion. Because of the long alkyl chains of the amine, the complex transfers to the organic phase[11]. At low concentrations of carboxylic acid, the acid-base stoichiometry in the complex (Alamine-carboxylic acid) is 1:1. At higher concentrations of acid, more then one carboxylic acid is taken up by the amine molecule. As seen in Fig. 6, this *overloading* is due to the formation of two hydrogen bonds – one between the acidic proton and the amine, and the other between the carbonyl group and an acidic proton of the second carboxylic acid (the hydrogen bonds are marked by doted lines).

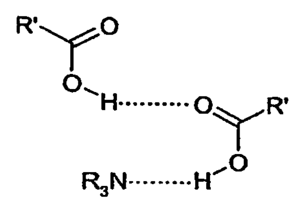


Fig. 6

The equilibrium of the carboxylic acid (HA) between the water and the organic phase is described in

Equation 1. Upon reaction with the tertiary amine (B), HA forms a complex (BHA) which remains largely in the organic phase. The efficiency of the extraction can be estimated by the distribution coefficient, $K_D = \frac{[BHA]_{org}}{[BHA]_{aq}}$.

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[8] Kertest, A. S. & King, C. J. (1986). Extraction chemistry of fermentation product carboxylic acids. *Biotech. Bioeng.* 28, 269-282.

[9] King, C. J. (1992). Amine-based systems for carboxylic acid recovery. *Chemtech.*, 285-291.

[10] San-Martin, M., Pazos, C. & Coca, J. (1992). Reactive extraction of Lactic acid with Alamine 336 in the presence of salts and lactose. *J. Chem. Tech. Biotechnol.* 54, 1-6. San-Martin, M., Pazos, C. & Coca, J. (1996). Liquid-liquid extraction of lactic acid with Alamine 336. *J. Chem. Tech. Biotechnol.* 65, 281-285.

[11] It is assumed that the excess of nitrous acid (which is by far the strongest acid at the media) is neutralized by the addition of strong inorganic base, and that the pH of the solution is below the pKa of the various hydroxy acids.

$$HA_{(aq)} + B_{(org)} \rightleftharpoons [BHA]_{(org)}$$

Equation 1

Of the various hydroxy-carboxylic acids, the extraction of l-lactic acid (LA) is the one investigated most intensively because of the various uses of LA in the pharmaceutical, cosmetics and polymer industry. The vast amount of data known about the extraction of LA on industrial scale, makes it an excellent model system for the extraction of various hydroxy-carboxylic acids. Examples of some distribution coefficients for LA are listed in Table 1[10]. It should be noted that in every case, the lower the initial pH, the grater the efficiency of the extraction[11].

Organic solvent	Amine	[LA] at equilibrium	T (°C)	K_D
Chloroform	Diethylbutylamine ($0.97\ mol\ dm^{-3}$)	0.97 M	25	1.80
Chloroform	Tributylamine ($0.97\ mol\ dm^{-3}$)	0.97 M	25	1.40
Chloroform	Triamylamine ($0.97\ mol\ dm^{-3}$)	0.97 M	25	2.70
Chloroform	Trioctylamine ($0.97\ mol\ dm^{-3}$)	0.97 M	25	4.50
Toluene	Alamine 336 ($0.4\ mol\ dm^{-3}$)	< 0.4 M	25	0.83
		< 0.4 M	50	0.81
Toluene	Alamine 336 ($0.8\ mol\ dm^{-3}$)	< 0.4 M	25	2.06
		< 0.4 M	60	1.06

Table 1

Choudhury et. al.[12] studied the extraction of LA with two extractants – trioctyl amine (tertiary amine) and Aliquat 336 (quaternary amine), in three diluents – methylisobutyl ketone, octanol and paraffin liquid. It has shown that tertiary amine is a better extractant then the quaternary amine. Among the three diluents, methylisobutyl ketone is the better solvent, probably due to it's higher polarity that better stabilizes the ionic complex of the amine and carboxylic acid.

The equilibria for extraction of hydroxy-carboxylic acids is affected by the nature of the organic solvent, the amine, the strength of the carboxylic acid (by means of pKa), the pH of the water and of course by temperature. Some of these factors can be used for the purpose of **back extraction** of the carboxylic acid from the extract back into water. This regeneration process is also known as *swing process*.

[12] Choudhury, B. & Swaminathan, T. (1998). Lactic acid extraction with trioctyl amine. *Bioprocess Eng.* 19, 317-320. Choudhury, B., Basha, A. & Swaminathan, T. (1998). Study of lactic acid extraction with higher molecular weight aliphatic amines. *J. Chem. Tech. Biotechnol.* 72, 111-116.

Temperature is the most useful factor by means of back extraction on industrial scale. As seen in Table 1, K_D for the extraction of lactic acid (when the organic phase is toluene and Alamine336 at 8 mol dm^{-3}) is reduced from 2.06 to 1.06 by elevating the temperature from 25°C to 60°C. Another example is the complexation constant of succinic acid with Alamine 336 (for the formation of complex at 1:1 molar ratio) which reduced by a factor of 10 when the temperature is elevated by 40°C. The organic phase with the amine and the rest of the acid-amine complex, is recycled by using the mixture for another extraction.

The process of back extraction is enhanced by using a mixture of organic solvents such as chloroform-heptane mixture. Larger proportions of chloroform in the mixture greatly enhance the extraction of the acid, because chloroform solvates the complex better. Distilling the chloroform before the back extraction (by elevating the temperature) will achieve a greater swing than is attainable with either approach alone. The chloroform (or any other "active" solvent) is added back to the mixture before it reenters the forward extractor.

Extraction with organo-phosphate compounds

Tributylphosphate, is a low-priced strong Lewis base which is very slightly miscible in aqueous phase (0.039 mass%), but highly soluble in organic solvents. These qualities are very important on large-scale production, where the pollution of the environment and the cost of reagents should also be taken into account. Malmary et al.[13] demonstrated the extraction of aconitic acid and lactic acid by the use of tributyl phosphate with pure dodecane as diluent, in application to the sugarcane industry.

In the case of a phosphorus-bonded oxygen donor extractant, the phosphryl group is a stronger Lewis base than traditional carbon-bonded oxygen donor solvents such as ketones and alcohols which give low partition coefficients with carboxylic acids. However, the relatively high viscosity of tributylphosphate $\left(3.56\times10^{-3}\,\mathrm{N\,s\,m^{-2}}\text{ at }298\mathrm{K}\right)$ and the fact that its specific gravity is close to unity (0.98) require the use of a diluent. Normally, polar diluents enhance the extracting power of the solvent but often are more soluble in the aqueous phase than non-polar compounds. Pure dodecane, has low viscosity $\left(1.15\times10^{-3}\,\mathrm{N\,s\,m^{-2}}\text{ at }298\mathrm{K}\right)$, low specific gravity (0.75) and is insoluble in water, thus it is an excellent diluent for tributylphosphate in liquid-liquid extraction system. Since dodecane is chemically inert with regard to the transfer of solute in organic phase, the percentage of tributylphosphate in the system 'extractant + diluent' should be as high as possible.

Extraction to a solid phase

An alternative route for the extraction of products from the reaction media, is the use of a solid phase with high affinity and high selectivity to hydroxy-carboxylic acids. The main advantage of the solid phase is that it can be separated from the liquid media simply by filtration and washings.

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[13] Malmary, G., Albet, J., Putranto, A., Hanine, H. & Molinier, J. (2000). Recovery of aconitic and lactic acids from simulated aqueous effluents of the sugar-cane industry through liquid-liquid extraction. *J. Chem. Tech. Biotechnol.* 75, 1169-1173.

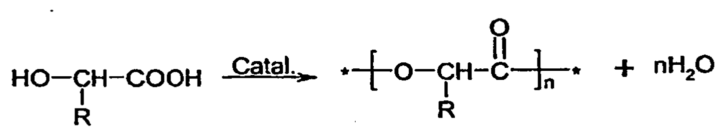
<u>Polymeric sorbents containing amine groups</u>: Basic amino groups attached to hard polymeric chain, in the form of microscopically particles, can serve as a stationary phase in a column. Passing the (neutralized) fermentation mixture through the column, will result in uptake of the acid by the basic stationary phase. The large surface area of the polymer beads (200 to 800 m^2/gr) allows the uptake of 0.3 gr acetic acid (for example) by 1 gr of dry polymer.

The same factors affecting the back extraction described in section 0 above, can be used here for the extraction of the acid back to the liquid phase. One additional and important method is leaching and washing the beads with another solvent. Washing with a basic aqueous phase is much effective then washing with neutral water. The problem of salt formation can be solved by the use of sufficiently volatile base like aqueous ammonia. The solution of the ammonium carboxylate salt is then concentrated and heated so as to decompose the salt, forming the acid product and ammonia for recycle. In some cases the ammonia can react with the carboxylic acid to form an amide, so a volatile tertiary amine like trimethylamine, which cannot form an amide, can be used.

Today, there are some commercially available basic sorbent like polyvinylpyridines, polybenzimidazoles and poly-*N*-oxides.

3.3. *Polycondensation of hydroxy-carboxylic protomers*

Polymerization of hydroxy-carboxylic protomers will take place by means of polycondensation reaction between the hydroxy and carboxyl groups to yield polyester bonds. Two of such polyesters are shown for (Fig. 7) poly(lactic acid) (PLA) and poly(glycolic acid) (PGA). Because of their biodegradability to nontoxic products, PLA and PGA are well established as very useful biodegradable polymers, covering a wide range of applications, such as dental, orthopedic and drug delivery. The scientific literature offers a vast amount of data and information on these polymers so they will be used here as a model system.



PGA, R=H
PLA, $R=CH_3$

Fig. 7

The process of polymerization is sensitive to various parameters that have influence not only on the course of reaction, but also on the properties of the final polymer such as molecular weight (Mw) and biodegradability.

Polymerization methods

Polymerization using cyclic diesters: Ring opening polymerization of cyclic diesters like lactide and glycolide, is the preferred method for producing high Mw polymers. Cyclic diesters can be synthesized from hydroxy-carboxylic acids using Sb_2O_3 as catalyst[14] (Fig. 8). At the first stage, low Mw polyester is obtained, and upon heating at low pressure, the glycolide or lactide is isolated by sublimation.

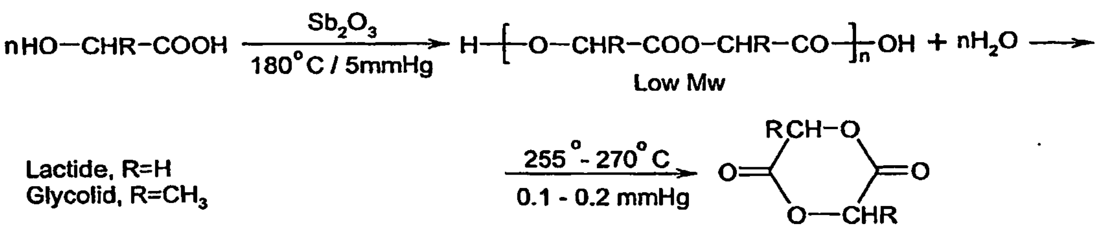


Fig 8

Polymerization of cyclic diesters is catalyzed by various catalysts like Sb, Zn, Pb, or preferably Sn. Stannous octoate (tin(II) salt of 2-ethylhexoic acid) is most suitable for the production of biodegradable polymer because of its acceptance by the FDA as a food stabilizer. According to Kohn et al.[15], the mechanism of the catalyzed ring opening polymerization is dependent on the catalyst that is used. The reaction (described schematically in Fig. 8) is preferably performed in the melt at high temperatures, or in solution under mild conditions.

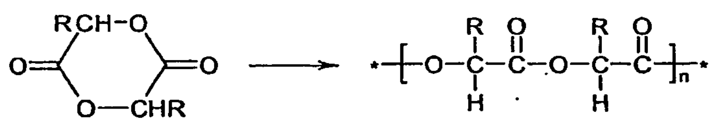


Fig. 8

For example, PLA, PGA and their copolymers where prepared with 0.03% stannous octoate and 0.01% lauryl alcohol (as a catalyst, activator, and chain control agent) at 220°C [15]. PLA was also obtained without the presence of alcohol and with concentration of catalyst in the range of 2×10^{-5} to 1×10^{-4} moles per mole of lactide[16].

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[14] Gilding, D. K. & Reed, A. M. (1979). Biodegradable polymers for use in surgery – polyglycolic / polylactic acid homo-and copolymers: 1. *Polymer* 20, 1459-1464.
[15] Kohn, F. E., Van Ommen, J. G. & Feijen, J. (1983). The mechanism of the ring-opening polymerization of lactide and glycolide. *Eur. Polym. J.* 19, 1081-1088.
[16] Eling, B., Gogolewski, S. & Pennings, A. J. (1982). Biodegradable materials of poly(L-lactic acid): 1. Melt-spun and solution-spun fibers. *Polymer* 23, 1587-1593.

Direct polymerization of hydroxy-carboxylic acids: Polyester can be obtained by direct condensation between the carboxylic and the hydroxy groups of the hydroxy-carboxylic acid (Fig. 9). Many methods are known today for performing the reaction and reviewing them all is beyond the scope of this paper. Some examples will be mentioned here, but it is clear that there are other well established methods reported in the scientific literature. The great advantage of the direct polymerization method, is that it saves the exhaustive purification of the cyclic diesters, and therefore makes the polymers less expensive. But, although simple, some polymers obtained by this method have low Mw.

Koyama et al.[17], prepared biodegradable polyesters by polycondensation of hydroxy-carboxylic acids derivatives. The hydroxy-carboxylic acids prepared from aminoalcohols and acid anhydrides, and then polymerized in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride $(EDC \cdot HCl)$ as a condensation reagent, and 4-dimethylaminopyridine (DMAP) as a catalyst. The result was biodegradable polymers with $\overline{M}_n = 9900 - 27200$.

Fig. 9

For example, the biodegradability of the polymer with $R^1 = -H$ and $R^2 = -CH_2CH_2-$ $(\overline{M}_n = 27200)$ was examined at 37°C in a phosphate-buffer (pH=7.8) solution. The $\overline{M}_n$ of the polymer decreased from 27200 to 400 after 72 h. meanwhile, when $R^2 = -CH = CH-$, the $\overline{M}_n$ of the polymer did not change at all under the same conditions.

Poly(hydroxy-carboxylic acids) can be made by direct polymerization of the linear hydroxy-carboxylic acid in the absence of catalyst, but usually, the polymers made by this method have low Mw (this is because the reaction conditions of high temperature and high vacuum can induce the formation of cyclic diester in equilibrium with the polymer). High Mw PGA was obtained by melt/solid poly-condensation of glycolic acid in the presence of various catalysts[18]. It is believed that the reaction has two step mechanism (Fig. 10) – in the first step, a low Mw oligomer is obtained which in the second step undergoes a chain-growth reaction.

The role of the catalyst is to enhance the dehydration rate of the oligomer without stimulating its depolymerization to cyclic diester (glycolide). The best catalyst was found to be Zn(II), and the second step is best performed in the solid state.

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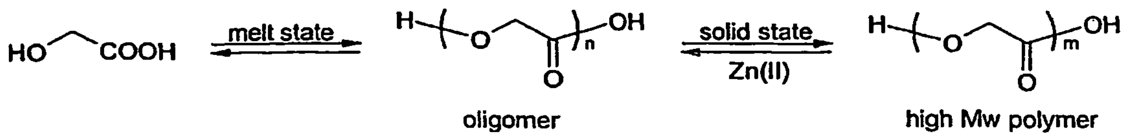

Fig. 10

Copolymerization of linear hydroxy-carboxylic acid with various lactones (γ-butyrolactone, δ-valerolactone and ε-caprolactone) was also preformed, in the absence of catalysts[19]. The reaction performed with 80-90% water solution of the monomer at 200°C under atmosphere of N_2. Because they remain in the polymer as end groups, initiators are generally undesirable for medical applications. Therefore, the polymerization of hydroxy-carboxylic acids in the absence of catalyst/initiator, is of great advantage.

Degree of cross linking

For linear polyesters such as polylactic polymer, one of the important conditions for obtaining a high Mw polymer, is keeping an equal unit ratio of the two functional groups taking part in the condensation. Even small amounts of carboxylic acids or alcohols cap the growing chains. In contrast, protein-derived polymers described in this invention produce branched cross-linked structures.

It is expected that many protomers will have more than one functional group (hydroxyl and/or carboxyl). It should be noted that the use of poly-functional monomers will have a direct affect on the degree of cross-linking in the polymer and therefore on its rate of degradation and mechanical properties. Obviously, highly cross-linked polymers will be degraded at a slower rate. The degree of crosslinking and the Mw will also affect the glass transition temperature, T_g, of the polymer. Highly cross-linked high-Mw polymers will have high T_g. The length and flexibility of the crosslinking arm will contribute to the lowering of T_g. In the total digest of an average protein source, the maximal degree of cross-linking is expected to be about 10%, as calculated from the ratio of Asp + Glu (carboxylates) to Lys + Ser + Thr (hydroxylates) in corn gluten and Soya been protein. Less digested protomers will offer a range of degrees of cross-linking

Post-polymerization modifications

In order to achieve the desired properties of the polymer, some modification can be made after the process of polymerization. Such modifications can alter the morphology, mechanical behavior, rate of degradation and many other characteristics of the polymer. Some examples will be discussed here briefly.

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[19] Fukuzaki, H., Yoshida, M., Asano, M., Aiba, Y. & Kaetsu, I. (1988). Direct copolymerization of L-lactic acid with δ-valerolactone in the absence of catalysts. *Eur. Polym. J.* 24, 1029-1036. Fukuzaki, H., Yoshida, M., Asano, M., Aiba, Y. & Kumkura, M. (1990). Direct copolymerization of glycolic acid with lactones in the absence of catalysts. *Eur. Polym. J.* 26, 457-461.

Incorporation of additives: One of the factors that influence the process of polymer degradation is the incorporation of additives. The effect of inorganic compounds such as calcium phosphate, sodium bicarbonate, and calcium carbonate is complex and changes from one polymer to the other. For example, Ara et al.[20], investigated the effect of calcium compounds on the degradation of PLA and its copolymer with glycolic acid. The additives (from the weakly acidic to the weakly basic) are calcium di-hydrogen phosphate, calcium hydrogen phosphate, calcium phosphate and calcium carbonate. Composite materials where made by adding powders of the inorganic compound (30% by weight of polymer) to a 10% solution of the polymer in dioxane, and then the dispersion was cast and frozen. Nonporous films with a thickness of 0.5mm, were made by compression at 60°C and $50\,\mathrm{kg/cm^2}$. The degradation of the various film types was studied in phosphate buffered saline (pH 7.4) at 37°C. It was found that the degradation of polymer decreased with increasing basicity of the calcium compounds blended. However, even the most acidic additive (calcium di-hydrogen phosphate), had appreciable degradation-delaying effects compared with unfilled copolymer.

Preparation of micro-spheres: The micro-encapsulation process in which the removal of the hydrophobic polymer solvent is achieved by evaporation has been widely reported in recent years for the preparation of micro-spheres and microcapsules based on biodegradable polymers and copolymers of hydroxy-carboxylic acids[21] (and references therein). In the solvent evaporation process, the polymer is dissolved in a suitable water immiscible solvent, and the medicament is dispersed or dissolved in this polymeric solution. The resultant solution or dispersion is then emulsified in an aqueous continuous phase to form discrete droplets. In order for the micro-spheres to form, the organic solvent must first diffuse into the aqueous phase and then evaporate at the water/air interface. As solvent evaporation occurs, the micro-spheres can be obtained after suitable filtration and drying.

The solvent evaporation method has been used extensively to prepare PLA and PLGA micro-spheres containing drugs (used as drug delivery systems). Many types of drugs with different physical and chemical properties have been formulated into polymeric system, including anti-cancer drugs, narcotic agents, local anesthetics, steroids, and fertility control agents.

Melt-spun and solution-spun fibers: In order to be suitable as implants for orthopedic surgery or blood vessels, polymers have to be biodegradable (so they will be replaced by the body's tissues), non toxic, and sufficiently strong to maintain their stability during the healing process. Fibers of PLLA (for example) are suitable for that purpose, but a maximum tensile strength at break of fibers with relatively low Mw, was in the range of 0.02-0.5 GPa. In order to improve their mechanical properties, solution-spun and melt-spun fibers with a high degree of molecular orientation and crystallinity were produced[17]. These fibers show better tensile properties – 0.5 and 1.0 GPa for the melt-spun and solution-spun, respectively.

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[20] Ara, M., Watanabe, M. & Imai, Y. (2002). Effect of blending calcium compounds on hydrolytic degradation of poly (DL-lactic acid-co-glycolic acid). *Biomaterials* 23, 2479-2483.
[21] O'Donnel, P. B. & McGinity, J. W. (1997). Preparation of microspheres by the solvent evaporation technique. *Adv. Drug Deliv. Rev.* 28, 25-42.

4. Biodegradability of Protein-Derived Proteins

Protein-derived polyesters consist of polyester-bonded peptidic protomers. Both kinds of bonds are biodegradable. The products of degradation are natural l-amino- and l-hydroxy-carboxylic acids.

The biodegradability of polymers is influenced by various parameters, which include the composition of the polymer, the polymerization conditions and, consequently, the morphology of the polymer (amorphous and crystalline). the storage history, etc. For example, the hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids was studied extensively lately[22]. The rate of degradation has to be adjusted according to the application that the polymer is about to fulfill, and as will be described in the following paragraphs. It is possible to synthesize poly(hydroxy-carboxylic acids) with different rate of degradation.

The degradation process is catalyzed by the carboxyl end groups. Since the cleavage of an ester bond yields a carboxyl group the process is auto-catalysed. At the very beginning, degradation occurs in the bulk and is macroscopically homogeneous. Later, soluble oligomers are generated in the matrix, and those that are close to the surface can escape from the matrix before total degradation. Oligomers inside the matrix, that can hardly escape, causing a higher acidity inside then at the surface. Therefore, autocatalysis is larger in the bulk then at the surface leading to surface-interior differentiation. Finally, hollow structures are formed when the internal material, which is totally transformed to soluble oligomers, dissolves in the aqueous medium.

The morphology of poly(hydroxy-carboxylic acids)[23], plays a critical role in the degradation process. It is now known that that degradation of semi-crystalline polyesters in aqueous media occurs in two stages. The first stage consists of water diffusion into the amorphous regions with random hydrolytic scission of ester bonds. The second stage starts when most of the amorphous regions are degraded. The hydrolytic attack then progress from the edge toward the center of the crystalline domains. Therefore it is obvious that amorphous polymers undergoes faster hydrolytic degradation.

Schwach et al. examined the influence of catalyst type on the hydrolytic degradation of poly(DL-lactide)[24]. It was found that PLA polymerized with Zinc-metal as initiator are much more hydrophilic, and consequently exhibit a faster molecular weight decrease, in contrast to PLA that polymerized with stannous octoate.

The composition of polymer chains greatly determines the degradation rate. For example, the half-life in terms of the weight loss of PLA was found to be longer then that of copolymers of lactic acid and glycolic acid. The main reason for that difference is that "pure" PLA is more crystalline the its copolymers.

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[22] Li, S. (1999). Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. *J. Biomed. Mater. Res. (Appl. Biomater.)* 48, 342-353.

[23] Cohen, D., Younes, H. & Marom, G. (1987). Amorphous and crystalline morphologies in glycolic acid and lactic acid polymers. *Polymer* 28, 2018-2022.

[24] Schwach, G., Coudane, J., Engel, R. & Vert, M. (2002). Influence of polymerization conditions on the hydrolytic degradation of poly(DL-lactide) polymerized in the presence of stannous octoate or zinc-metal. *Biomaterials* 23, 993-1002.

As described above, it is possible to modify the plastic by incorporating acidic or basic compounds in the polymeric matrix. If the compound is acidic, it can accelerate the degradation of the polymer. In contrast, for basic compounds, two effects can intervene simultaneously: base catalysis and neutralization of carboxyl end groups. Whether the degradation is accelerated or slowed down depends on the relative importance of the two effects. The addition of basic additives can also cause to homogenous degradation of the polymer as the case with coral granules.

The influence of Mw can be anticipated from the autocatalytic degradation mechanism. In fact, the higher the Mw, the lower the carboxyl end group concentration and the slower the degradation, at least at the early stages. Thus, the purification process to eliminate monomeric and oligomeric impurities is of great importance since they lead to a rapid degradation.

The size and shape of polymer devices is also an important factor. If the size is very small, the degradation should be slower then large-sized devices because in the former case, no auto-catalytic degradation occurs because of the easier diffusion of oligomers and neutralization of carboxyl end groups by the aqueous media.

Claims:

1. A process of producing a biodegradable plastic comprising:
deaminating undigested protein, protein fragments, small peptides, amino acids or mixtures thereof with nitrous acid or with nitrogen oxides, so as to replace most or all of alpha-amino groups, omega-amino group of lysine and to some extent guanidinic group of arginine with hydroxyls; and
poly-condensing hydroxy-carboxylic protomers resulting from said deaminating to form the biodegradable plastic.

2. The biodegradable plastic resulting from the process of claim 1.

3. A polymer of the following structure: